Nuclear Magnetic Resonance and Conformational Energy Calculations of Repeat Peptides of Elastin. Conformational Characterization of Cyclopentadecapeptide *cyclo*-(L-Val-L-Pro-Gly-L-Val-Gly)$_3$

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Received September 2, 1980

Abstract: Proton magnetic resonance and conformational energy calculations are presented for the cyclic peptide *cyclo*-(VPGVG)$_3$ containing the pentamer repeat of elastin. The ^{1}H NMR spectrum of the molecule is obtained in methanol and the temperature dependence of the peptide NH protons are reported for this solvent over the range 0-60 °C. The observation of a pentamer spectrum, at all temperatures, demonstrates that the molecule has a threefold symmetry on the 220-MHz time scale. ABX analysis is done for the Gly CH_2 spin systems and the ^3J vicinal and ^2J geminal coupling constants are derived. From the observed coupling constants, acceptable ranges of values for the torsion angles, ϕ, about N-C$^\alpha$ bonds are worked out. Reasonable ranges of values for torsion angles, ψ, about the C$^\alpha$-C bonds are gleaned from wire models built by using threefold symmetry and secondary structural constraints. By varying the backbone torsion angles within the ranges expected from coupling constants and from molecular models, it was possible to obtain a set of exactly threefold symmetric conformations. Conformational energy calculations are carried out, followed by detailed energy minimization. Six minimum energy structures are fully described. The torsion angles of three of these are consistent with NMR data. One of the structures is quite similar to the crystal structure of this molecule.

There are three repeat sequences that are found in tropoelastin, namely, -L-Val$_1$-L-Pro$_2$-Gly$_3$-Gly$_4$- (VPGG), -L-Val$_1$-L-Pro$_2$-Gly$_3$-L-Val$_4$-Gly$_5$- (VPGVG), and -L-Ala$_1$-L-Pro$_2$-Gly$_3$-L-Val$_4$-Gly$_5$-L-Val$_6$- (APGVGV).[1,2] In a series of previous publications from this laboratory,[3-6] structural studies on these elastin repeat peptides using NMR techniques in combination with conformational energy calculations have been reported. The structural studies have resulted in the identification of the dominant conformation(s) in solution adopted by linear peptides of these sequences. The proposed conformations contain interesting secondary structural features: (a) An $O_1 \cdots N_4$ hydrogen bond forming a 10-atom ring with -L-Pro$_2$-Gly$_3$- segment at the corner of a type II β turn in the linear tetramer, pentamer, and hexamer in the solvents Me_2SO, MeOH, $CHCl_3$, and water, (b) an $N_3 \cdots O_5$ hydrogen bond forming an 11-atom ring of a γ-turn in the penta- and the hexapeptide with a good probability of occurrence in Me_2SO, and (c) an $N_1 \cdots O_4$ hydrogen bond forming a 14-atom ring in the tetramer and pentamer under certain conditions of solvent system and temperature.

Cyclic peptides of these sequences have been particularly fascinating. They are also relevant since they may exhibit conformational features quite similar to those of their linear counterparts under suitable conditions. This point of view, known as the concept of cyclic peptides with linear correlates, has already been discussed.[7-10] Of course, some cyclic peptides will have uniquely constrained conformations. Examples of this are *cyclo*-(APGVGV)$_2$ where it is not expected that the linear polymer (APGVGV)$_n$ will be its linear correlate[7] and *cyclo*-(VPGVG) which is not a conformational correlate of the linear polypentapeptide.[10,11]

Various cyclic peptides of the pentamer repeat *cyclo*-(VPGVG) have been synthesized and their NMR properties relating to secondary structure have been systematically studied.[10] Detailed comparison between the set of cyclic peptides with n = 1, 2, 3, 4, 5, and 6 with the linear polypentapeptide shows a remarkable similarity between the NMR spectra of polypentapeptide and that of *cyclo*-(VPGVG)$_n$ with n = 3, 4, 5, and 6. In particular, the cyclic peptides *cyclo*-(VPGVG)$_3$ and *cyclo*-(VPGVG)$_6$ have NMR properties almost identical with that of the linear polypentapeptide.[10] Furthermore, an X-ray study of the crystal structure of the cyclopentadecapeptide *cyclo*-(VPGVG)$_3$ has been recently reported.[12] In the light of these studies, the conformational characterization of *cyclo*-(VPGVG)$_3$ by NMR and energy calculations becomes a meaningful undertaking of current interest.

Materials and Methods

The cyclic pentadecapeptide $\overline{\text{(L-Val}_1\text{-L-Pro}_2\text{-Gly}_3\text{-L-Val}_4\text{-Gly}_5)_3}$ was synthesized in this laboratory as outlined in an earlier article.[10] Deuterated methanol, CD_3OD, was purchased from Thompson Packard, Inc. while CD_3OH was purchased from Merck Sharp and Dohme of Canada. The complete deuteration of the peptide NH protons was achieved by dissolving the *cyclo*-(VPGVG)$_3$ in deuterated water and then drying the sample down under high vacuum. Approximately a 10 mM solution of this cyclic peptide was made in both the CD_3OD and CD_3OH solvents.

Proton Magnetic Resonance Spectra. All ^{1}H NMR spectra were obtained on a Varian HR-220 spectrometer operating at 21 °C unless otherwise mentioned. So that the ABX-spin spectral analysis of Glycyl methylene protons could be facilitated, simulated spectra were obtained with the SS-100 computer system by using a Varian Data Machine spin simulation program.

Valuable information regarding possible ranges of torsion angles may be obtained from an analysis of the observed coupling constants.[13]

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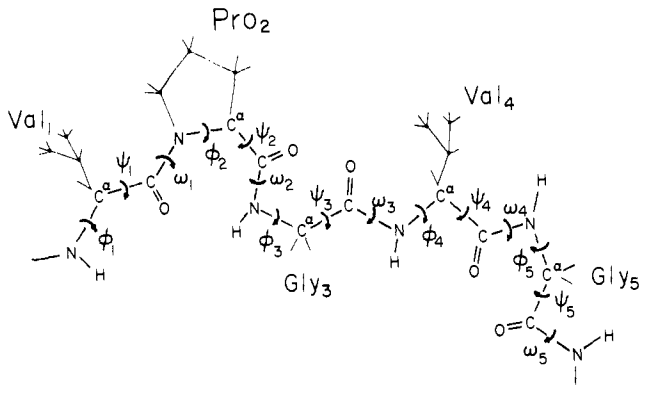

Figure 1. Schematic drawing of the pentamer sequence -*VPGVG*- showing the backbone torsion angles ϕ_i,ψ_i.

Ranges of the ϕ torsion angle of the valine residues were obtained from experimentally derived $^3J_{NH-C^\alpha H}$ coupling constants with use of a Karplus-type equation[13,14] as modified by Bystrov and co-workers,[15] i.e., eq 1, where θ is related to ϕ by $\theta = |\phi - 60|$ for L-amino acids. Similarly,

$$^3J_{NH-C^\alpha H} = 9.4 \cos^2 \theta - 1.1 \cos \theta + 0.4 \quad (1)$$

for the ϕ torsion angle of the glycine residues, eq 2 of Bystrov and co-workers[15] is employed.

$$^3J_{\alpha CH-NH} + {}^3J_{\alpha CH'-NH} = -9.8 \cos^2 \phi - 1.3 \cos \phi + 15.0 \quad (2)$$

An estimate of the ranges for the ψ torsion angle of the glycine residues is obtained from the observed values for the geminal couplings, ^2J, with use of an expression given by Barfield et al.[16] (eq 3).

$$^2J_{\alpha CH'-\alpha CH}(\phi,\psi) = -13.91 - 1.55 \cos^2 \psi - 2.80 \cos^4 \psi + 4.65 \cos^2 \phi \quad (3)$$

Finally, from the observed temperature coefficient ($\Delta\delta/\Delta T$) of the NH proton chemical shifts, a qualitative description of the extent of shielding of the NH protons is derived. This yields useful clues regarding the intramolecular hydrogen bonds present in the molecule, with the assumption, of course, that intramolecular hydrogen bonding is the source of shielding of the NH protons from the solvent molecules. In this regard in previous extensive studies on the repeat elastin peptides, delineation of both shielded NH and CO moieties were utilized to identify the particular hydrogen bonding responsible for solvent shielding.[6]

Conformational Energy Calculations. The purpose of the energy calculations presented here is to systematically explore the conformation space available for this cyclic molecule, to identify all the types of stereochemically reasonable conformations of the molecule that are consistent with the NMR coupling constant data, to refine these structures by energy minimization with a view to obtain detailed descriptions of the low-energy structures, and to compare these acceptable conformations with the solid-state conformation of the molecule already established from X-ray studies.[12] Several assumptions made in the calculations may be noted here. (a) Solvent effects are entirely ignored. (b) The molecule is assumed to exhibit perfect threefold symmetry. NMR data suggest the existence of this symmetry on the NMR time scale. Threefold symmetry is also retained in the crystal structure where the threefold axis coincides with one of the crystal axes.[12] (c) The entire conformation space geometrically available for the molecule is not searched. Instead, the search is limited to within the ranges of torsion angles deduced from NMR. While considerably reducing computing time, this eliminates much of the unnecessary analysis of structures totally incompatible with the coupling constant data. Thus an ab initio deduction of all possible conformations is not intended. The calculations, therefore, enable derivation of conformations by blending structural information deduced from NMR with well-known stereochemical rules governing the stability of a cyclic peptide. Energies are calculated following the method of Scheraga and co-workers[17,18] with bond lengths, bond angles, parameters of the nonbonded potential function and the atomic partial charges given by them.[17] However, computer programs developed in this laboratory are used. The proline ring closure is achieved by specifying the value of Γ, which is the pseudorotation of the C^γ atom about the C^β---C^δ line.[19] The self-energy of the proline ring is obtained by using the procedure described elsewhere.[19]

The procedure adopted here for obtaining low-energy structures may be described by the following sequence of steps.

Step 1. Coarse Search of Conformation Space. The principal degrees of freedom of a polypeptide chain backbone are the internal rotations, ϕ and ψ, about the N-C^α and C^α-C^β bonds[20,21] (Figure 1). With use of the method of Go and Scheraga[22,23] to obtain cyclic conformations with exact threefold symmetry,[23] two torsion angles have to be adjusted to achieve cyclization. Therefore, out of a total of 30 ϕ,ψ torsion angles, the threefold symmetry reduces the number of angles to be varied to 10, and the condition of cyclization further limits this number to 8. In the initial coarse search, it is quite reasonable to limit the value of ϕ_2(Pro$_2$) to -60°. Thus, only seven independent variables are involved. NMR analyses reported here result in permissible ranges of values for the torsion angles, ϕ (see Table II below). No such information is deduced for the angles ψ. However, it is known that the range of acceptable values for ψ_1 of Val$_1$ is limited by the interaction between the valyl and prolyl side chains in the Val-Pro segment. Thus, steric hindrance between these side chains can be avoided only if ψ(Val) lies in the range ~60–150°. The value of ψ_2 of Pro$_2$ is also limited[24] to a very narrow interval at ~-60° and another range from 100 to 180°. An estimate of the limitations on the angles ψ_3 and ψ_4 imposed by cyclization was obtained by examination of a Kendrew wire model built with the other torsion angles retained within permitted ranges. With the choice of ϕ_5,ψ_5 at Gly$_5$ as the dependent angles to ensure threefold symmetric cyclization, with use of standard, constant bond lengths and bond angles,[17,18] and with ϕ_2 held fixed at -60°, the seven independent angles ϕ_1, ψ_1, ψ_2, ϕ_3, ψ_3, ϕ_4, and ψ_4 were varied in steps of 20° within the ranges obtained from NMR and steric considerations. For the resulting set of angles, the solution of cyclization with threefold symmetry was attempted. The remaining set of angles leading to successful threefold symmetric cyclization resulted in a set of conformations of the cyclic molecule. This formed the set of "starting" structures from which low-energy structures are to be obtained.

Step 2. Energy Calculations for the Set of "Starting" Structures. Conformational energies are computed for the set of structures synthesized in the first step. This is essentially a pruning step where the backbone conformations with severe steric conflicts are to be discarded. In order to make this selection without prejudgement of the detailed nature of the side-chain conformations, valyl side-chain atoms beyond the C^β and H^α are ignored in calculating the energies. Also, the proline ring is kept in the standard "down" γ-puckered structure. Wherever severe steric hindrance was encountered primarily involving the prolyl atoms, the calculation was repeated with the "up" γ-puckered ring structure. This step results in the identification of the low-energy regions worthy of further consideration.

Step 3. Exploration of Neighboring Conformations by Lattice Search. This stage consisted of a systematic step-wise exploration of the seven-dimensional conformation space on a 10° grid in the low-energy regions already identified. Energy calculations are done for a cluster of conformations in the seven-dimensional lattice. Valyl side chains are omitted as before. A set of low-energy conformations is obtained.

Step 4. Complete Energy Calculation. Total conformation energy is computed for each structure selected with all side-chain atoms included. In general, all the three ideal valyl side-chain conformations with χ^1 = 60, 180, and -60° are considered. All the methyl hydrogens are held at perfectly staggered conformations. Both "down" and "up" puckered proline ring structures are considered. Only low-energy conformations are retained.

Step 5. Complete Energy Minimization. Starting from each of the low-energy conformations, an energy minimization is performed with use of a quasi-Newton algorithm by Fletcher.[25] In the initial cycles of refinement, all the eight backbone torsion angles ϕ_1, ψ_1, ..., ϕ_4, ψ_4 and the proline ring parameter Γ were allowed to vary. Of course, the solution of cyclization conditions was always in effect, thus constantly

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Table I. ^{1}H NMR Parameters of *cyclo*-(VPGVG)$_3$ in Methanol

PARAMETERS	Val_1	Pro_2	Gly_3[a]	Val_4	Gly_5[a]
Chemical Shifts (δ)[c] in ppm (± 0.01)					
δ-NH	8.21	-	8.50	7.98	8.43
δ-$C^{\alpha}H$	4.18	4.27	3.67,4.12	4.43	3.83,4.02
δ-$C^{\beta}H$	2.0-2.3	1.8-2.3	-	2.0-2.3	-
δ-$C^{\gamma}H$	0.86-1.13	2.0-2.3	-	0.86-1.13	-
δ-$C^{\delta}H$		3.70-3.50	-	-	-
Coupling Constants (J) in Hz (± 0.25)					
$^3J_{(NH-C^{\alpha}H)}$	8.5	-	4.5,7.0	10.0	5.5,6.0
$^3J_{(C^{\alpha}H-C^{\beta}H)}$	9.0	b	-	9.0	-
$^3J_{(C^{\beta}H-C^{\gamma}H)}$	7.0	b	-	7.0	-
$^2J_{(HH)}$	-	b	-17.0	-	-17.0
Temperature Coefficient ($\frac{\Delta\delta}{\Delta T}$) x 10^{-3} in ppm/°C					
Peptide NH	-10.0	-	-5.8	-3.8	-8.0

[a] Values were obtained by ABX spin analysis of Gly CH_2 protons. [b] Not analyzed. [c] Me_4Si used as internal standard.

adjusting the values of ϕ_5,ψ_5 to preserve cyclization. In the later stages of the minimization, the following parameters were also allowed to vary: (a) all the six ω torsion angles, thus allowing peptide units to become nonplanar; (b) all the five $\tau(C^{\alpha})$, the bond angles at C^{α} atoms; (c) χ^1, $\chi^{2,1}$, $\chi^{2,2}$ of both valyl side chains permitting rotations about C^{α}–C^{β} and C^{β}–C^{γ} bonds of valines. The minimization iteration was terminated when the decrease in energy per iteration became less than 0.01 kcal/mol.

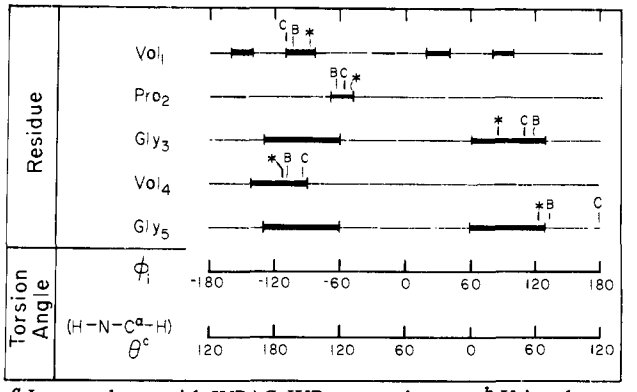
Results

Proton Magnetic Resonance Spectra. The 220-MHz ^{1}H NMR spectrum of *cyclo*-(VPGVG)$_3$ in CD_3OH is shown in Figure 2A ($C^{\alpha}H$ region only). Figure 2B shows the spectrum of the same molecule in CD_3OD but devoid of $^3J_{NH-C^{\alpha}H}$ couplings by preexchanging the peptide NH protons with deuterium (see Materials and Methods). By comparing these two spectra (Figures 2A and 2B) and by spin-decoupling, the resonances of valines, prolines, and glycines were readily assigned. The delineation between the Val_1 and Val_4 and between the Gly_3 and Gly_5 resonances was tentatively made by following the spectral behavior of Gly_3 and Val_4 resonances. It has been well documented that the -L-Pro_2-Gly_3- fragment, in elastin peptides particularly, induces a type II β turn.[6] Occurrence of this β turn imparts a large nonequivalence of the Gly_3 methylene (CH_2) protons and more shielding of the Val_4 NH proton. It can be seen in Figure 2 that the methylene protons (CH_2) assigned to Gly_3 are almost 0.5 ppm apart while the Gly_5 CH_2 protons are 0.19 ppm apart (see Table I). The temperature dependence of all the peptide NH protons is also listed in Table I where it could be seen that the peptide NH proton assigned to Val_4 is the most shielded one (-3.8×10^{-3} ppm/°C). The ^{1}H NMR parameters such as chemical shifts and coupling constants obtained in methanol are given in Table I.

That the molecule, *cyclo*-(VPGVG)$_3$, possesses a C_3 symmetry within the NMR time scale and that there are no cis–trans isomerizations within detection limits of a few percent are indicated by the observation of single resonance for each proton and for a group of chemically equivalent protons. This can be seen particularly in the NH proton region depicted in Figure 2C. The conformational characteristics of this molecule could, therefore, be fully described by the spectral properties of the repeating unit VPGVG. In contrast to this unique feature, *cyclo*-(VPGVG) shows multiple conformations in the proton NMR spectrum.[10]

Analysis of $^3J_{NH-C^{\alpha}H}$ coupling constants by employing eq 1 and 2 gives ranges of various torsion angles, ϕ. These are depicted in Table II. Although there is yet no well-established NMR method available to determine the torsion angle, ψ, the nuclear Overhauser effect (NOE) has been successfully used by several workers[26-28] and by us in this laboratory on the elastin peptides[5,7,29] to obtain an estimate of the angle, ψ. Unfortunately, in this study

Table II. Ranges of ϕ_i[a] Derived from $^3J_{NH-C^{\alpha}H}$(▬)[b]



[a] In accordance with IUPAC-IUB nomenclature. [b] Using the Bystrov equations (see the text). Asterisk denotes angles found in the crystal structure, while B and C are the angles of the minimum energy structures B and C (Figure 3). [c] $\theta = |\phi - 60|$ for L-amino acids.

Table III. Confromational Torsion Angles of *cyclo*-(VPGVG)$_3$

RESIDUES	TORSION ANGLES	RANGE[a]	CONFORMATION[b] WITH γ-TURN	CONFORMATION[b] WITHOUT γ-TURN
Val_1	ϕ_1	-180 to -80	-100	-95
	ψ_1	100 to 140	130	130
Pro_2	ϕ_2	--60	-60	-55
	ψ_2	100 to 160	120	145
Gly_3	ϕ_3	60 to 120	120	90
	ψ_3	-60 to +40	-50	-20
Val_4	ϕ_4	-140 to -100	-105	-115
	ψ_4	30 to 120	70	105
Gly_5	ϕ_5	-180 to -60, 85 to 180	130	125
	ψ_5	30 to 180, -180 to -55	140	155

[a] Ranges of angles leading to acceptable 3-fold symmetric cyclic conformations. [b] Torsion angles from model building (see text).

Table IV. Minimum-Energy Conformations of the Cyclopentadecapeptide[a]

	STRUCTURE		A	B	C	D	E	F	CRYSTAL[d]
	TOTAL ENERGY		7.1	8.2	8.3	8.3	8.6	9.5	-
BACKBONE TORSION ANGLES	(kcal/mole/pentamer)								
	Val_1	ϕ	-131	-106	-112	-125	-107	-100	-91
		ψ	93	127	115	100	124	124	129
		ω	178	175	172	173	173	173	177
	Pro_2	ϕ	-52	-63	-58	-55	-51	-55	-53
		ψ	146	119	118	146	116	126	140
		ω	177	-173	-180	180	-170	177	175
	Gly_3	ϕ	85	119	111	93	128	84	84
		ψ	-74	-52	-58	-65	-49	42	-7
		ω	-179	170	-176	180	-178	-178	-176
	Val_4	ϕ	-131	-106	-95	-130	-86	-138	-111
		ψ	119	73	95	116	100	117	119
		ω	177	-169	178	178	176	180	174
	Gly_5	ϕ	-151	131	-180	-133	-149	-144	122
		ψ	-99	139	-178	-150	66	76	179
		ω	174	-174	179	178	-176	-179	-178
SIDE CHAIN TORSION ANGLES	Val_1[b]	$\Delta\chi^1$	4	0	2	-1	1	-1	
		$\Delta\chi^{2,1}$	1	3	-3	-3	2	-1	
		$\Delta\chi^{2,2}$	9	8	7	-1	7	5	
	Pro_2[c]	Γ	25	-9	13	20	20	16	
	Val_4[b]	$\Delta\chi^1$	-1	4	0	-3	2	-4	
		$\Delta\chi^{2,1}$	-3	-3	-4	-5	2	-5	
		$\Delta\chi^{2,2}$	7	8	7	6	7	9	
BOND ANGLE DEFORMATIONS AT Cα ATOMS		$\Delta\tau_1$	2	-4	1	-2	1	-1	3
		$\Delta\tau_2$	-6	-7	-6	-5	-8	-7	1
		$\Delta\tau_3$	8	0	3	5	4	6	7
		$\Delta\tau_4$	4	2	1	3	0	7	-1
		$\Delta\tau_5$	-5	-8	-5	-5	-6	-5	4
SELECTED N···O DISTANCES		$O_1\cdots N_4$	4.0	3.2	3.4	3.8	2.9	3.7	3.3
		$N_3\cdots O_5$	8.6	2.9	6.6	8.0	7.7	9.1	
		$N_1\cdots O_4$	5.9	3.9	4.4	5.1	4.8	6.5	

[a] All angles are rounded off to the nearest degree and the energies to the first decimal place. The recommendations of IUPAC-IUB[20] are followed regarding nomenclature and conventions. [b] The valyl side chains are in the trans conformation with the torsion angle χ^1(N-C^α-C^β-$C^{\gamma 1}$) ~180°. $\Delta\chi^1$ is the change in χ^1 from the perfectly trans conformation. $\Delta\chi^{2,1}$ and $\Delta\chi^{2,2}$ are similarly departures of γ-methyl hydrogens from the perfectly staggered conformation about the C^β-C^γ bonds. [c] Γ is the pseudorotation of the C^γ atom about the $C^\beta\cdots C^\delta$ line. See ref 19. [d] From reference 12, included here for the convenience of comparison.

at 100 MHz, the large OH signal of CD_3OH masked the $C^\alpha H$ proton resonances of interest such that no experimental verification of ψ was possible. However, Table III contains gross estimates of ϕ and ψ as obtained from the Kendrew wire model built on the basis of the experimentally derived ranges of ϕ and the formation of intramolecular H bonds (i.e., the β turn and the γ turn).

Minimum Energy Conformations. With use of planar peptide units and standard bond lengths and bond angles, the molecule readily cyclizes with threefold symmetry and with torsion angles lying in the ranges depicted in Table II. In fact, 30% of the set of angles considered in step 1 lead to successful solution of cyclization conditions. The ranges of backbone torsion angles consistent with a threefold symmetric cyclic structure are listed in Table III, from which it can be seen that this cyclic molecule is quite flexible. The possible values of ϕ,ψ at Gly_5 fall into two separate ranges. Therefore one may expect to find at least two quite different types of conformations.

The six best minimum energy conformations obtained are shown in Figure 3 where their stereo ORTEP plots are given. All the geometric parameters completely describing these minimum energy structures are listed in Table IV. The values of N···O distances of interest, found in each of these structures, are also given in the table.

Discussion

Proton Magnetic Resonance Spectra. Since the molecule involves the repeating unit VPGVG, it would be interesting at this point to consider the spectral characteristics of this unit in its linear monomeric form in methanol and in chloroform. Chloroform, being a nonpolar solvent, promotes the formation of H bonds, rendering the molecule less flexible in solution and thus making

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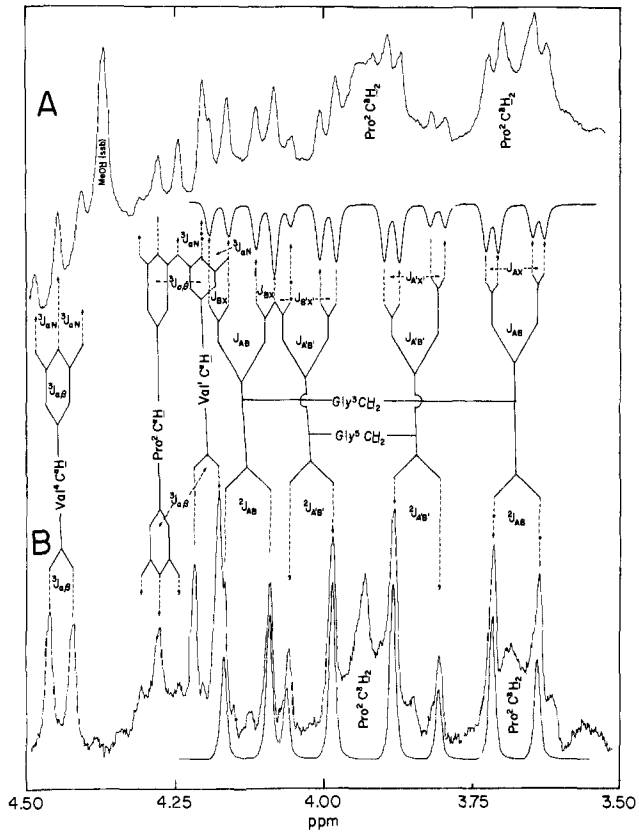

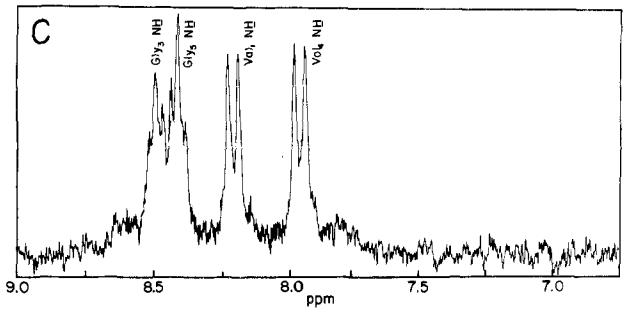

Figure 2. 220-MHz ^{1}H NMR spectrum of *cyclo*-(VPGVG)$_3$: (A) in methanol-d_3 and (B) in methanol-d_4. Note exact match of the computer simulated spectrum obtained by ABX spin analysis for Gly$_3$ and Gly$_5$ C$^\alpha$H$_2$ protons. (C) Peptide NH proton region in methanol-d_3.

the linear molecule more comparable with the cyclic counterpart. Methanol, however, being a polar solvent would destabilize some of the inter- and intramolecular interactions. Such solvent effects and the molecular flexibility can be evidenced in Table V which contains the temperature dependence of peptide NH protons of VPGVG in the linear and cyclic forms. In both solvents and both forms, the Val$_4$ NH proton is most shielded while the Val$_1$ NH and Gly$_5$ NH protons are completely exposed. Interestingly, however, the Gly$_3$ NH proton shows a different behavior which needs further explanation as described below. In the polypenta- [(VPGVG)$_n$] and polyhexa- [(APGVGV)$_n$] peptides of elastin there occurs a γ turn, an 11-membered H-bonded ring between Gly$_3$ NH and Gly$_5$ C=O groups. If such a turn occurs, one would then expect to observe a higher shielding of Gly$_3$ NH proton, as evidenced by a decreased temperature dependence of chemical shift. In the case of linear VPGVG in $CDCl_3$, a relatively higher shielding (-5.1×10^{-3} ppm/°C) for the Gly$_3$ NH proton was observed (see Table V) while in MeOH this proton was largely exposed (-7.5×10^{-3} ppm/°C). It was, therefore, implied that over 50% of the time the γ turn exists in chloroform whereas it

Table V. Temperature Dependence of Peptide NH Protons (Expressed in ppm/°C)

RESIDUE	HCO-VPGVG-OMe		Cyclo-(VPGVG)$_3$
	MeOH[a]	$CDCl_3$[b]	MeOH
Val$_1$ NH	0.0086	0.0068	0.0100
Gly$_3$ NH	0.0075	0.0051	0.0058
Val$_4$ NH	0.0048	0.0038	0.0038
Gly$_5$ NH	0.0079	0.0093	0.0080

[a] From ref 6. [b] From ref 4.

is much less probable in methanol. In *cyclo*-(VPGVG)$_3$, on the other hand, the Gly$_3$ NH proton is not totally exposed ($\Delta\delta/\Delta T = -5.8 \times 10^{-3}$ ppm/°C; see Table I and V) which indicates either the presence of a weak γ turn or a γ turn that exists at least for some fraction of the time.

The presence of a γ turn induces nonequivalence of the Gly$_5$ CH$_2$ protons as does the β turn to the Gly$_3$ CH$_2$ protons, and the degree of nonequivalence depends on the ring constraint imposed by the H-bonded structure as was observed in *cyclo*-(APGVGV)$_2$.[7] Examination of the nonequivalence of glycyl CH$_2$ protons in *cyclo*-(VPGVG)$_3$ reveals that, while Gly$_3$ CH$_2$ protons show the chemical shift difference of almost 0.5 ppm, the Gly$_5$ CH$_2$ protons show a difference of only 0.19 ppm. This, therefore, indicates that the β turn is well stabilized as could also be seen from the temperature dependence of the Val$_4$ NH proton (see Table I and V), whereas the small chemical shift difference of Gly$_5$ CH$_2$ protons could either be attributed to the cyclization constraint or to the formation of a γ turn for a certain fraction of time. The conformation of *cyclo*-(VPGVG)$_3$ could, henceforth, be described by taking into account the well-stabilized β turn together *with* and *without* a γ turn.

A Kendrew wire model of *cyclo*-(VPGVG)$_3$ was constructed with a β turn (type II) formed by a H bond between the Val$_4$ NH and Val$_1$ C=O groups. The $^3J_{NH\text{-}C^\alpha H}$ coupling value of 8.5 Hz for the Val$_1$ residue gives rise to four possible ranges of ϕ torsion angle (see Table II). A value of $\phi_1 = -95$ seems to be satisfactory in the wire model. With this value of ϕ_1 and with the Val$_1$ C=O H bonded to the Val$_4$ NH proton, a value of $\psi_1 = 130$ describes the model well. With the ϕ_2 torsion angle of Pro$_2$ being locked at -55 and with ψ_2 at 145, the β turn can be constructed.

However, the ϕ_3 and ψ_3 angles of the Gly$_3$ residue at the other corner of the β turn will vary depending on whether or not the γ turn with the H bond between Gly$_3$ NH and Gly$_5$ C=O groups is formed. The sum of ^3J coupling values of Gly$_3$ gives two broad regions of ϕ_3 (see Table II). A value of $\phi_3 = 120$ satisfies the wire model with the γ turn while ϕ_3 fixes well at 90 in absence of the γ turn. An analysis of Gly$_3$ CH$_2$ proton geminal coupling (^2J) of -17.0 Hz (Table I) utilizing the expression of Barfield and co-workers[16] gives the range of ψ_3 from 0 to ± 30 or from 180 to ± 150. Accordingly the perspective from the wire model is that the ψ_3 value changes from -50 to -20 when the γ turn is removed. The $^3J_{NH\text{-}C^\alpha H}$ coupling value of 10.0 Hz for Val$_4$ indicates only the negative range of ϕ_4 which at -105 satisfies the model with the γ turn but otherwise appears in the wire model to fit best at -115. The torsion angle ψ_4 similarly changes from 70 to 150 on breaking the 11-membered H bond (γ turn) in the wire model. On the basis of ABX pattern, Gly$_5$ shows a greater flexibility. An analysis of coupling parameters (^3J and ^2J) of Gly$_5$ (Table I) as done for Gly$_3$ (see above) gives the ranges of torsion angles for ϕ_5 and ψ_5. From the wire model with the γ turn, $\phi_5 = 130$ and $\psi_5 = 140$ which on releasing the γ turn changes to $\phi_5 = 125$ and $\psi_5 = 155$. These model-derived values fall well within the NMR-allowed ranges. After the stepwise description of the Kendrew wire model of *cyclo*-(VPGVG)$_3$ based on the experimentally derived ranges of ϕ_i values and H-bonding information had been completed, all the conformational torsion angles thus obtained were tabulated in Table III. It is appropriate to recognize here that only gross estimates of torsion angles can be obtained

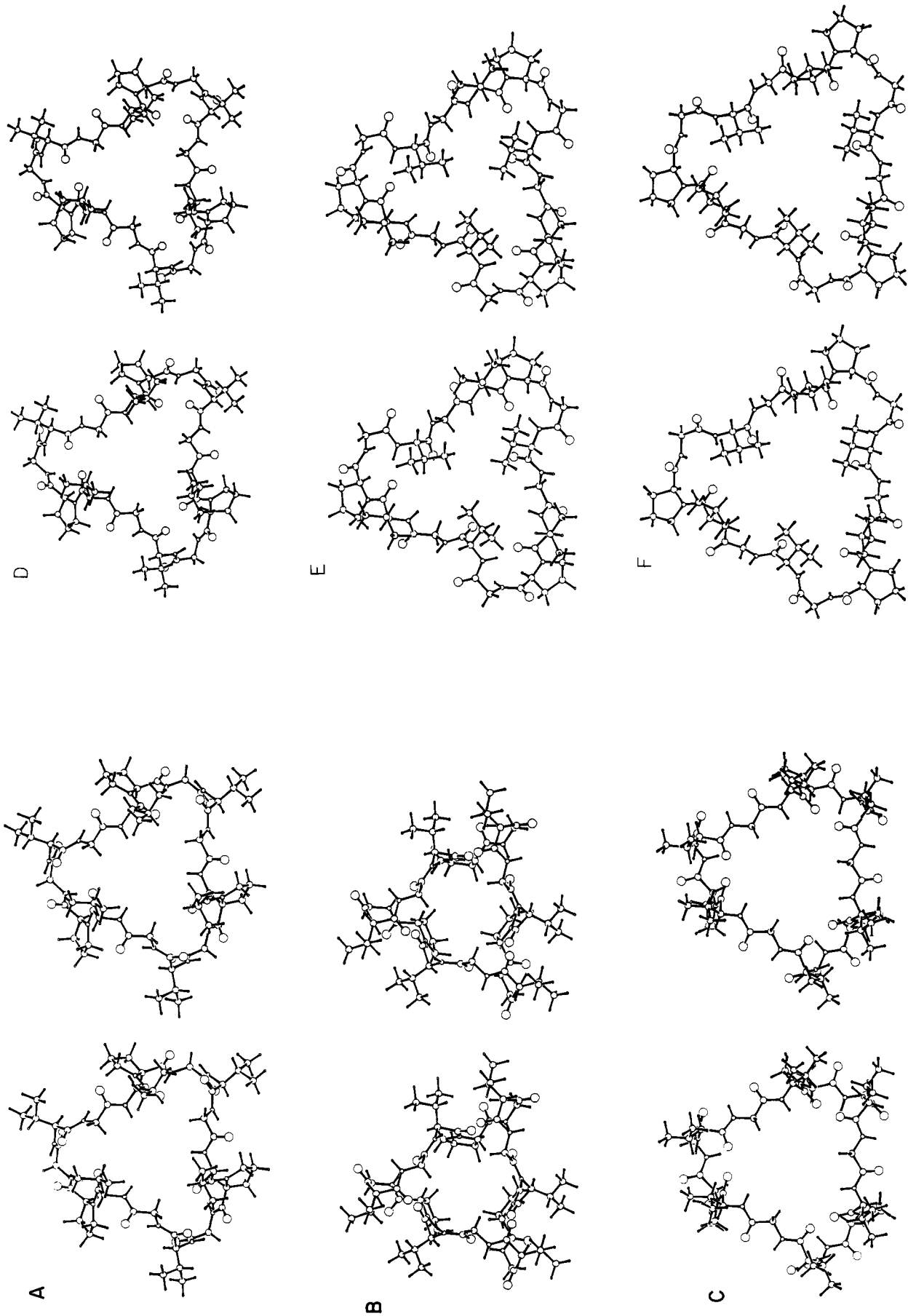

Figure 3. ORTEP stereoviews down the threefold axis of the six minimum energy conformations of *cyclo*-(VPGVG)$_3$.

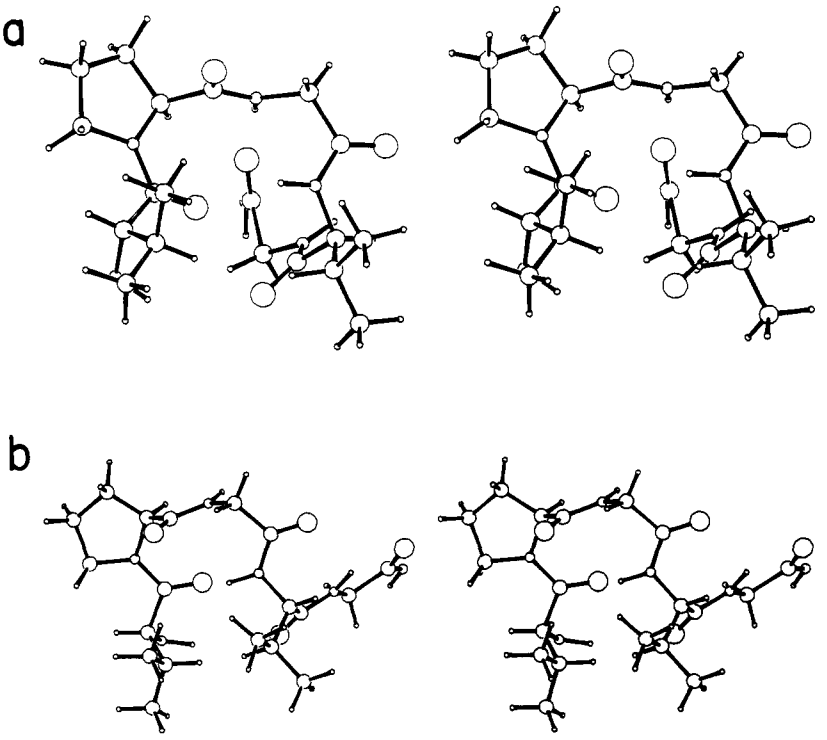

Figure 4. Stereoplots of the conformation of the pentamer segment -VPGVG- in minimum energy structures B and C shown in Figure 3: (a) structure B showing the β and the γ turn; (b) structure C containing only the β turn as in the crystal structure.

from such wire models. Relieving steric hindrance and adhering to strict threefold symmetry are achieved more efficiently by computer calculations. The values reported in Table III are, therefore, only rough estimates of the torsion angles.

Analysis of $^3J_{C^\alpha H-C^\beta H}$ for the valine residues gives side-chain orientations of Val_1 and Val_4 residues by employing the Abraham–McLaughlan expression.[30] A $^3J(\alpha\beta)$ value of 9.0 Hz for both the Val_1 and Val_4 residues (see Table I) indicates a slightly off trans conformation of their side chains or some flexibility about the trans orientation. There is no change in the side-chain orientation of the valine residues for the change in backbone conformation due to the absence or presence of the 11-membered H-bonded ring (γ turn). An interesting conformational feature of this molecule could be noticed in its torsion angles, listed in Table III, which shows some changes in the residues involved in γ turn but does not drastically change the overall conformational characteristics when the γ turn is ruptured. This is evident in the molecular model where it can be seen that the changes in torsion angles of Gly_3, Val_4, and Gly_5 residues on opening the γ turn result in a change in the distance between the Gly_3 NH and Gly_5 C=O groups from 3.0 to 5.6 Å (see, for example, Figure 4 below).

Minimum-Energy Structures. All the low-energy structures shown in Figure 3 appear to be stereochemically reasonable. All the interatomic distances are found to be satisfactory. Their energy differences cannot be considered to be significant. One may compare the torsion angles in these structures listed in Table IV with the acceptable ranges of ϕ angles (Table II) to examine the consistency of these structures to NMR data. It can be seen that ϕ angles of all the structures except C lie within acceptable ranges. The value of $\phi(Gly_5)$ in C is at $\pm 180°$, well outside the range stipulated by coupling constant data. Therefore, from the ranges given in Table II alone, it will be difficult to favor any one of these structures. Broadly speaking, the structures A, B, C, and D may be considered to belong to one class, with E and F to another, primarily on the basis of the value of angle $\psi(Gly_5)$. The difference between the two types is markedly seen in the ORTEP plots.

The structures E and F are quite unique in that Val_4 side chains tend to be oriented toward the inside of the cyclic system, giving the appearance of a hydrophobic interior. Actually when viewed in three dimensions it is seen that the Val side chains are on the top side of the molecule. Even so, this interesting situation is reminescent of the "oiled-coil" structure proposed for elastin-repeat peptides by Gray, Sandberg, and Foster.[2] In all the other structures shown in Figure 3, all the valyl side chains are directed toward the outside of the molecule.

The challenging realities of the experimental data are actually met. This is seen by comparing the degree of solvent exposure of NH protons, as interpreted from observed temperature coefficients of the peptide NH chemical shifts, with the intramolecular NH···O hydrogen bonding present in the low-energy static structures. As discussed earlier, the temperature dependence of NH proton chemical shift shows that in *cyclo*-$(VPGVG)_3$ in methanol the Val_4 NH proton is well shielded while the Gly_3 NH proton is only partially shielded. Thus, in methanol, the structure is expected to contain a β turn having the segment -Pro_2-Gly_3- at the turn with a good $O_1 \cdots N_4$ hydrogen bond. On the other hand, the partial shielding of Gly_3 NH indicates either that the structure contains a γ turn with a weak $N_3 \cdots O_5$ interaction or that the solution structure is a mixture of one containing a good γ turn and another without a γ turn. In the latter case, the structure may be dynamic, changing from one with a γ turn to one without it, each structure occurring for a certain fraction of the time. Now, structures B, C, and E contain the type II β turn at Pro_2-Gly_3. Only structure B shows a strong γ turn. While a mixture of structures B, C, and E can explain the temperature dependence of chemical shift, dynamic interconversion between E and the other two structures is unlikely since it will involve a large change in the torsion angle $\psi(Gly_5)$ associated with a large energy barrier. Thus it is reasonable to consider that a mixture of structures B and C may be present in methanol.

It is interesting to compare these low-energy structures B and C of *cyclo*-$(VPGVG)_3$ with those found for the linear pentamer. Since the previous report[4] on the linear pentamer employed a different set of energy functions, it is more appropriate to make the desired comparison with the low-energy conformation of the linear pentamer obtained with the use of potential functions employed in this paper. Table VI lists the torsion angles of the two low-energy conformations of the linear pentamer with use of the current potential functions. Structure A′ contains the type II β

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Table VI. Low-Energy Conformations of the Linear Pentamer Segment (VPGVG)

RESIDUE	TORSION ANGLES	STRUCTURES A'	B'
Val_1	ϕ	-118	-117
	ψ	131	103
Pro_2	ϕ	-53	-65
	ψ	110	115
Gly_3	ϕ	120	130
	ψ	-57	-70
Val_4	ϕ	-120	-97
	ψ	90	80
Gly_5	ϕ	100	102
	ψ	-175	-166

turn at Pro_2-Gly_3 and the $N_1 \cdots O_4$ hydrogen bond forming a 14-atom ring. Structure B', on the other hand, while containing the same β turn, shows also a γ turn with an $N_3 \cdots O_5$ hydrogen bond but not the shorter $N_1 \cdots O_4$ distance of a 14-atom ring. These structures are consistent with the NMR data on the linear pentamer.[4,6] Their torsion angles are not very different, and therefore interconversion between the two structures can easily take place. Structure B of *cyclo*-$(VPGVG)_3$ and B' of the linear pentamer seem to be similar, the essential alteration due to cyclization is seen in the value of the torsion angle ψ_5. Another effect of cyclization may be the inability to form the 14-atom ring as readily in the cyclopentadecapeptide, though this distinction is not particularly evident on comparison of the data on the cyclopentapeptide series with the linear polypentapeptide.[10] In both structures B and C, however, while the $N_1 \cdots O_4$ distance is too long to call a hydrogen bond, the orientation is favorable as seen in the β turn stereo perspectives of Figure 4.

The conformation of *cyclo*-$(VPGVG)_3$ found in the crystal structure[12] is generally similar to structure C derived here. The largest difference is found in the value of ψ_5, which is 122° in the crystal structure. As noted earlier, despite the cyclic nature, *cyclo*-$(VPGVG)_3$ is a rather flexible molecule with the largest flexibility being at the Gly_5 residue. The carbonyls of Val_4 are directed more toward the center of the molecule, in the crystal structure, facilitating interaction with water molecules found inside the cyclic peptide. These solvent interactions and the intermolecular interactions present in the crystal lattice are ignored in the present calculations. In view of these, the degree of agreement between the structure C and the crystal structure is quite satisfying.

The torsion angles of the crystal structure are listed in Table IV. The ϕ angles in the crystal are also indicated in Table II, from which it can be seen that the crystal structure angles all fall well within the allowed ranges obtained from solution NMR coupling constant data.

While the general perspective of structure C approximates that of the crystal, the angles of low-energy structure B may be seen to be quite close to those of the solid-state conformation. Thus, with small changes in torsion angles, one can readily go from structure B to the crystal structure; in this process while the γ turn is ruptured, the size of the central cavity is also increased in order to accommodate the water molecules found in the crystal structure.

Acknowledgment. This work was supported in part by the National Institutes of Health, Grant No. HL-11310 and GM-07195.

The Role of Adsorption in the Initial One-Electron Electrochemical Reduction of Nicotinamide Adenine Dinucleotide (NAD^+)

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Abstract: The title reduction was investigated at pH 9.1 in several base electrolytes of varying surface activity in order to elucidate the role of adsorption of NAD^+, its free radical, and the resulting dimer at the aqueous solution/mercury electrode interface. In the presence of electrolytes of low activity, NAD^+ is adsorbed at potentials positive to its 1-e reduction (ca. -0.9 V vs. SCE); the electrochemically generated dimer, $(NAD)_2$, is adsorbed positive of -1.20 and -1.32 V in 0.06 and 0.4 M KCl solutions, respectively. NAD^+ undergoes both diffusion- and adsorption-controlled reduction; the former predominates on slow time scale experiments and the latter on fast time scales. From low concentration surfactant (0.06 and 0.1 M tetraethylammonium (Tea^+) chloride) solutions, NAD^+ is only adsorbed positive of -0.65 V and an adsorption-controlled prewave appears, indicating that an adsorbed layer of NAD· and/or $(NAD)_2$ is formed on reduction of dissolved NAD^+. From a high concentration (0.4 M) Tea^+ solution, NAD^+ is adsorbed positive of -0.66 V, but the adsorption-controlled prewave is suppressed and the reduction is entirely diffusion controlled. Under diffusion control, the heterogeneous rate constant for the title reduction is ca. 0.1 cm s^{-1} and the rate constant for dimerization of NAD· is ca. 3×10^6 M^{-1} s^{-1}.

β-Nicotinamide adenine dinucleotide (NAD^+; Figure 1) is a high-energy coenzyme of great biochemical importance. In numerous biological hydrogen-transfer reactions, it accepts two electrons and a proton from a substrate (SH_2) in the presence of a suitable enzyme, forming 1,4-dihydronicotinamide adenine dinucleotide (1,4-NADH; Figure 1), the oxidized form of the substrate (S) and a proton.

$$NAD^+ + SH_2 \xrightleftharpoons{\text{enzyme}} \text{1,4-NADH} + S + H^+ \quad (1)$$

The nicotinamide ring is the site of the reversible hydrogen transfer which is stereospecific with respect to both substrate and nicotinamide ring.

Several aspects of the electrochemical behavior of the NAD^+/NADH redox couple have been investigated and reviewed.[2] Cathodic reduction of NAD^+ occurs in two discrete

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